

("CV"); and 3) reduced inter-site CV. The improved image quality is due to the fact that, without speckle, the fluorescence images give a very clear picture of the morphology of the reaction sites. The intra-site intensity variation may be significantly reduced and better reflect the actual reaction site morphology, thus eliminating the need for averaging over a large spatial area in order to obtain a reliable average reaction site signal intensity. Accordingly, it may be possible to reduce the size of the reaction site and pack more features into the microarray without suffering from speckle-induced degradation in the data quality. Furthermore, in the presence of speckle effects in the captured image, the spatial averaging in a fluorescence image affected by speckle is not ideal, even with a reaction site spot diameter of ~0.6 mm. In an array with replicate features within a row, the speckle therefore contributes to the inter-site CV. Rotating diffuser 1270 may substantially eliminate the speckle-induced contribution resulting in an improved inter-site CV.

[0117] Referring again to FIGS. 15-17, diffuser 1270 is placed in the excitation beam in the abovementioned target imaging system. Diffuser 1270 may be rotated, for example, using a motor 1271. In an example, the diffuser has random structure which imposes structure on the laser beam by means of diffraction. The diffuser does not significantly degrade the coherence of the laser beam. However, if the laser beam is passed through rotating diffuser 1270 (rotating in the plane of the diffuser about an axis offset from the beam axis), any speckle effect is virtually eliminated from the laser beam profile when time-averaged over a sufficiently long time period. Consequently, when the microarray imaging exposure time is much greater than the diffuser structure sampling time, all signs of speckle in the excitation light disappear. This provides uniform excitation of the microarray, which in turn yields speckle-free fluorescence images.

[0118] Referring particularly to FIG. 18, a diagrammatic illustration of an exemplary waveguide-based sample imaging system 1800 is shown, in accordance with an embodiment. A laser diode 1802 emits a diverging beam 1804, which is passed through a collimating lens 1806 so as to be transformed into a collimated beam 1808. Collimated beam 1808 passes through a diffuser 1820, which is rotated about an axis parallel to the propagation direction of collimated beam 1808, so as to generate a uniform beam 1810. Uniform beam 1810 is directed toward a waveguide arrangement 1830. Waveguide arrangement 1830 includes an integrated lens 1832, a planar portion 1834 and an assay region 1836, which includes a plurality of reaction sites 1838. Reaction sites may include immobilized biomolecules such as antigens, antibodies, proteins, peptides, glycans, or nucleic acids. Various methods of preparing printed microarrays, including contact printing, inkjet printing, piezoelectric printing, and solenoid valve jet printing are available.

[0119] Continuing to refer to FIG. 18, uniform beam 1810 is coupled into waveguide arrangement 1830 via integrated lens 1832 such that uniform beam 1810 is guided through planar portion 1834 by total internal reflection. Consequently, the evanescent wave portion of uniform beam 1810, so guided through planar portion 1834, illuminates assay region 1836. Any resulting signal, such as fluorescence, is captured at a sensor 1840. This method may be applicable for both a waveguide with an integrated coupling lens (as shown in FIG. 18) and a waveguide with coupling optics that is separate from the planar portion of the waveguide arrangement.

[0120] The location of the rotating diffuser in the path of collimated beam 1808 is advantageous because rotating diffuser 1820 may be selected to impart only a small amount of divergence to collimated beam 1808 while generally preserving the coherence of collimated beam 1808. In other words, the diffuser may be disposed as close to planar portion 1834 such that any small, potentially unavoidable divergence of collimated beam 1808 created by diffuser 1820 has minimal effect over the extent of waveguide arrangement 1830. Also, for combinations of a small laser diode and an inexpensive collimating lens, space constraints may preclude the possibility of inserting a diffuser in the path of diverging beam 1804.

Imaging System

[0121] Referring again to FIGS. 12-14, an imaging sensor (such as a two dimensional CMOS or CCD sensor) may be used in reader instrument 1200. In one embodiment illustrated in part with respect to FIGS. 12 through 14, sensor 1212 may be mounted in reader instrument 1200. Imaging optics may be positioned over the imaging sensor. Use of imaging optics fixed with respect to the imaging sensor may lower reader instrument cost, eliminate a requirement for potentially expensive autofocus circuitry, and increase tolerance of the sensor to shock.

[0122] The sensor may be, for example, MT9M001 from Micron (Aptina), which is a 1/2", 1.3 Mpixel, monochrome sensor with 1280×1024 pixels (Pixel size: 5.2 μm×5.2 μm). The objective system may be, for example, V13VM615 from Xiamen Leading Optics, which is a closed-circuit television ("CCTV") lens with variable focus/zoom/iris with f/1.4 and focal length of 6-15 mm. The objective system may be used as is or, alternatively, separated into components and mounted into a custom-made barrel. Due to the fact that the imaging system creates a demagnified image in performing the analyte detection, the depth of field of the aligned system is approximately 2 mm. The object to image distance may be, for example, less than 100 mm. A fluorescence emission filter may be placed within the imaging system, in an embodiment. The fluorescence emission filter serves to block light having approximately the same wavelength as the laser light illuminating the planar waveguide, while transmitting fluorescence signal from the assay region. For example, the fluorescence emission filter may be positioned between the objective system and the cartridge, or between separated components within the objective system. The resulting magnification provided by the imaging system is approximately 1/5. The resulting pixel size in object space is approximately 35 μm×35 μm. The light signal is prevented from reaching a vertical belt (short dimension) along one side of the sensor such that this part of the sensor then gives a measurement of the dark noise (i.e., readout noise). Furthermore, a series of exposure times are used in the imaging process in order to extend the dynamic range to almost 4 orders of magnitude, as enabled by the dark noise subtraction.

Example 2

Automatic Cartridge Identification with Peripherals

[0123] Accurate cartridge identification and tracking is useful for commercial reader instruments. Peripheral tracking modules based on one or two dimensional bar codes, RFID readers, or other conventional tracking technologies are contemplated. As may be seen in FIG. 19, for example, a